A COMPARATIVE STUDY OF THE EFFICACY OF THE VARIOUS AGAR-DYE-MEDIUMS RECOMMENDED FOR THE ISOLATION OF TYPHOID AND DYSENTERY BACILLI FROM FECES

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Introduction

While investigating peptic and tryptic digests as substrata for culture mediums in routine work, we found that some of these preparations regularly gave better growth of the organisms belonging to the typhoid-dysentery group than the ordinary veal- or beef-infusion or extract-mediums.

This immediately suggested the possibility of improving the existing methods for the detection and isolation of B. typhosus and B. dysenteriae or paradysenteriae. As the result of a few preliminary tests, however, it was quite evident that only a careful comparative study of the existing differential mediums would supply sufficient information to permit of reliable conclusions. The ever increasing reports on elaborate mediums, for the detection of typhoid and dysentery bacilli in stools, show clearly that the problem of this particular technic is not yet solved. And inasmuch as the control of enteric fever and of dysentery will largely depend on the diagnosis and bacteriologic supervision of potential carriers, the knowledge and the application of reliable methods which regularly permit the isolation of the infecting organisms is absolutely necessary even when their numbers are very few. In fact, in experimental work with typhoid carriers in rabbits and other animals, we have encountered difficulties which were traceable to the unreliability of the solid culture mediums used for the detection of typhoid baccilli in the dejecta. The progress of our studies on carriers and dysentery infections depended largely on the development of one or two methods which rendered the isolation of the offending organisms a comparatively simple process.

It is for these reasons that we have retested a large number of recently described methods. In presenting the results, we wish to

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point out why negative and misleading findings are frequently obtained by the use of some mediums. At the same time we wish to discuss the advantages of peptic-liver or tryptic beef-heart digests as substrata in the dye-mediums to be applied for the isolation of typhoid and dysentery bacilli. We wish further to demonstrate the relative merits of the various mediums and the immediate purpose for which they are applicable.

Too little attention has been paid to the latter point in connection with the isolation of dysentery bacilli. And, as will be pointed out, the customary assumption that most mediums used for the detection of typhoid bacilli are also suitable for the isolation of dysentery organisms is incorrect. In examining cases of infantile dysentery, in studying the persistence of dysentery bacilli in the feces of convalescence, and in searching for dysentery carriers, we have met with constant disappointment in the results obtained by using Endo's medium (original formula) or litmus lactose agar. Furthermore, certain observations made us suspect that some of the substances needed as indicators in the mediums inhibited or destroyed the bacilli sought for. Later studies have clearly shown that when relatively few bacilli are present, for example, in the formed stools of convalescents, they are frequently not demonstrable on these mediums. The improvements which we suggest are preliminary to investigations which have been in progress for the last two years, and they give promise of producing a selective medium for dysentery bacilli.

For the sake of clearness it appears to be advisable to consider separately our experiments with the various methods and compositions of mediums applicable for the isolation of typhoid and dysentery bacilli.

I. THE DETECTION AND IDENTIFICATION OF THE ORGANISMS OF THE TYPHOID-PARATYPHOID GROUP BY MEANS OF SELECTIVE MEDIUMS

It has been the general experience that most of the mediums recommended for the detection of typhoid bacilli in stools are more or less inhibitory and that small points of preparation or handling impaired materially the final result. Laboratory workers seem to get the best results usually with the medium to which they are most accustomed. The general use of Endo medium in all the institutes which are handling a great deal of typhoid material is, in our opinion, sufficient proof

of the efficacy of this method. Dreyer, Walker and Gibson[1] as the result of a series of tests of the three differential mediums (MacConkey, Drigalski-Conradi, Endo) in common use concluded in a recent publication that Drigalski-Conradi medium was the least efficient, and Endo decidedly the best. Tidy and Dunn[2] confirmed these observations, but did not find such great differences between the MacConkey and Endo medium. We agree with their statement that, as a rule, more colonies always develop on the ordinary plain agar than on the selective medium. It is unfortunate that Dreyer, Walker and Gibson fail to state the method of preparing the various mediums they submitted to the careful tests which they report. Their results would gain considerably in value if this valuable information had been presented more completely. The MacConkey medium, even though more inhibitive than the Endo medium, has the great advantage of indicating the nonlactose fermenting organisms with striking clearness, which enables the picking out of pure colonies far better than the Endo medium. This particular defect of the latter medium is particularly marked when a soft agar substratum and when a faintly alkaline reaction to litmus is chosen.

Robinson and Rettger[3] tried to overcome the diffusion of the indicator color by recommending a reliable modification of the Endo medium. In our experience their modification is excellent for separating and differentiating typhoid or paratyphoid organisms, when relatively abundant, from a variety of other organisms by selective elimination. But we have observed, as will be shown, that when the typhoid-paratyphoid bacilli are relatively few in numbers, the use of this Endo modification may be misleading as regards the value of a negative finding in a stool examination. We fully agree with Holt-Harris and Teague[4] that the reaction of the Endo medium when adjusted to —0.2 (in our experience a P^H of 7.8-84.4) is too alkaline to permit the optimal growth of the typhoid bacillus, and that this condition limits the usefulness of this type of medium for primary isolation. The modification of Kendall[5] produces less inhibition of growth, but on account of the rapid diffusion of the restored dye through the medium from the B. coli colonies, heavy seeding of the plate is impossible and the picking of colonies is made rather laborious.

[1] Lancet, 1915, 1, p. 643.
[2] Jour. Roy. Army Medical Corps, 1916, 27, p. 482.
[3] Jour. Med. Research, 1916, 34, p. 363.
[4] Jour. Infect. Dis., 1916, 18, p. 600.
[5] Jour. Med. Research, 1911-1912, 25, p. 95.

In recent years these disadvantages have led to the development of selective mediums in which the differentiation of the nonlactose from the lactose fermenting colonies is accomplished by the use of neutral red, or Congo red, or water blue, or china blue as indicators. With the exception of the Congo-red medium of Schmitz,[6] our experiments have shown that these mediums are mostly inferior to the Endo medium. The addition of these dyes to plain lactose agar provokes considerable inhibition of the typhoid bacillus, and does not permit the optimal growth of these bacteria. China blue and water blue in combination with other dyes give, as we intend to demonstrate later, selective mediums of considerable value.

An entirely new method of differentiating nonlactose-fermenting organisms from fecal bacteria has been devised by Holt-Harris and Teague in their eosin-methylene blue agar. Aside from having very little inhibitory action, the medium differentiates typhoid from coli bacilli with most remarkable distinctiveness. No diffusion of the color takes place and the medium can be set to the optimum reaction necessary for the typhoid-paratyphoid bacillus without changing the indicators.

Since Loeffler, and later Conradi, have found that malachite green and brilliant green solutions, respectively, have a marked restraining action on B. coli, numerous solid and liquid mediums have been devised which inhibit the growth of many strains of B. coli and other fecal bacteria to a much greater extent than they inhibit B. typhosus or paratyphosus. Among the many attempts of Lentz and Tietz, Loeffler, Conradi, Fawcus and others to prepare selective mediums with brilliant green or malachite green, only two of the recently described methods, the medium of Krumwiede, Pratt and McWilliams[7] and one of Teague and Clurman[8] deserve further consideration.

The observations of these writers have not as yet been confirmed, and extensive comparative studies with these mediums as regards their practical utility have not been published.

From some of our test experiences we were at once convinced, however, that the eosin-brilliant green medium of Teague and Clurman is the most satisfactory preparation as yet recommended for the isolation of typhoid bacilli from stools. The medium of Krumwiede has certain disadvantages which we shall discuss later.

[6] Deutsch. med. Wchnschr., 1915, 41, p. 426.
[7] Jour. Infec. Dis., 1916, 18, p. 1.
[8] Ibid., 1916, 18, p. 647 and p. 653.

The only objection to the continuous use of Teague's medium in our laboratory, aside, perhaps, from the difficulty in obtaining the proper stains, was the employment of expensive veal infusion peptone agar as a substratum for the dyes. Meat extract preparations also proved unsatisfactory, but the introduction of peptic digests resulted, as already indicated, in the preparation of a cheap, selective medium which surpassed all our expectations. In using such digests the Teague-green medium has been slightly improved and represents in our opinion in this form the only reliable, selectively inhibitive and differentiating medium to be used for typhoid and paratyphoid detection from feces.

After this brief discussion of the status of the methods and their modifications used for the detection of typhoid-paratyphoid bacilli, we desire to present our results under two general captions.

(1) The value of the various indicators, inhibitive dyes, etc., employed in the agar substrata so far as they are outlined in the original descriptions of the methods.

(2) Methods of improving these mediums by use of peptic digests as substrata.

We have[9] called attention to the value of the peptic digests, but in conjunction with our work on the typhoid bacillus the following points deserve particular emphasis.

1. Peptic digests of pig liver and stomach supply amino-acids at an extremely low cost. They furnish complex nitrogenous compounds, which, as Gordon[10] has recently determined, are essential growth factors for the B. typhosus. In this respect they are superior to tryptic digests of beef or casein and the addition of "nutrose" to the extract or infusion agar, which has been recommended by many workers, is superfluous.

2. The dextrose and bile salts in the liver digests are substances which are known to be stimulating to typhoid bacilli. In this connection it should be recalled that Krumwiede as well as Teague[11] have recommended the addition of dextrose to some of their mediums.

3. Digests of blood-clots are equally well suited for typhoid mediums, if one of the processes described in our previous communications is followed. The addition of serum, as Schmitz[12] has suggested, is of

[9] Jour. Infect. Dis., 1918, 22, p. 68.
[10] Jour. Royal Army Med. Corps, 1917, 28, p. 371.
[11] Jour. Med. Research, 1917, 35, p. 107.
[12] Centralbl. f. Bakteriol., I, O., 1915, 76, p. 306.

advantage in connection with such clot digests. In our experience peptic liver digests are less troublesome to make and are therefore to be preferred.

Before giving figures for some of the quantitative experiments, it will be of interest to briefly outline our method of study.

METHODS OF STUDY

The various selective mediums to be tested were carefully prepared according to the original formula, or according to one of the recently recommended modifications. Of the various formulas of special mediums, which we have chosen for quantitative tests, the following are to be mentioned: Endo's medium, original method;[13] modification of Kendall;[5] modification of Robinson and Rettger;[3] Drigalski-Conradi medium;[14] Congo-red agar;[15] malachite-green-china blue agar of Bitter;[16] eosin-brilliant green of Teague;[8] brilliant green agar according to Krumwiede,[7] methylene blue-eosin agar according to Holt-Harris and Teague; MacConkey's medium,[17] and Gonzalez agar.[18]

Particular attention was paid to all details and the various steps given in the descriptions of the methods were faithfully adhered to. Before pouring the plates or before adding the dyes the reaction of the agar medium was determined by one of the colorimetric methods used for the determination of the H-ion concentration. In the experiments with peptic digest substrata, the P_H was adjusted to the same reaction as was determined in previous experiments to be the optimum. This P_H reaction frequently corresponded well with the one of the original method, but differed, for well known reasons, from the titrable reaction. For brilliant green mediums the optimum reaction is—according to numerous tests—P_H 7.0-6.8. Shohl and Janney[19] recently found the maximum growth of B. typhosus (Rawlings) to be at P_H 6.4; our studies showed some variations, the majority of strains grew best, however, at a P_H 7.0-6.4.

All the other peptic digest mediums, with the exception of the Endo's medium, were adjusted to P_H 7.0-7.2. The mediums were poured in constant amounts (25 c c) into large petri-dishes of 12 cm. diameter. As a rule the prepared dishes were dried for 15 minutes in a large incubator (especially installed for this purpose) and used immediately or stored for a few hours. Plates older than 18 hours were not used in these experiments.

Experiments were made by preparing:

(1) Artificial stools.

(2) Known mixtures of pure cultures of B. typhosus and B. coli. High dilutions of these were spread on the surface of the mediums to be tested, and the number of colonies of the two organisms which grew on the plain agar was compared with the number of colonies obtained from the same quantity of the same dilution on the surface of plates of the various special mediums studied.

[13] Centralbl. f. Bakteriol., I, O., 1904, 35, p. 109.
[14] Ztschr. f. Hyg. u. Infectionskrankh., 1902, 39, p. 283.
[15] Deutsch. med. Wchnschr., 1915, 41, p. 427.
[16] Centralbl. f. Bakteriol., I, O., 1911, 59, p. 469, and 1913, 71, p. 228.
[17] Jour. Hyg., 1908, 8, p. 322, and Thompson-Yates Lab. Rep., 1901, 3, p. 151.
[18] Semaine méd., 1913, 32, p. 574.
[19] Jour. Urol., 1917, 1, p. 211.

(3) Suspensions of specimens of stool from well known typhoid carriers (rabbits and man) and also from convalescents from typhoid fever or dysentery were spread in known dilutions on the surface of the test mediums.

Numerous experiments with artificial stools did not permit of any quantitative expression of the results, but they confirmed the observations made with stools from carriers, and these are therefore not tabulated.

For the quantitative studies, the method used by Dreyer, Walker and Gibson was followed. The dilutions were prepared from 24-hour old peptic digest broth cultures and the platings were always made with 0.02 c c of the diluted culture or mixture measured by means of accurate, certified serologic pipets. The fluid drop was carefully spread over the surface of the agar by means of a bent 15 gauge "nichrome" wire. The plates were incubated for 24 hours at 37 C.

The preparation of stool suspensions followed the customary procedures; one 2 mg. loopful of the broth or bile suspension was inoculated on each plate and spread by means of the bent wire.

EXPERIMENTAL DATA

Series 1.—In a series of experiments the veal infusion Witte's peptone-agar as a substratum in Endo's, Congo-red, Drigalski-Conradi, eosin-brilliant green and malachite green-china blue medium was compared with peptic digest agar containing the same dyes and indicators. For further comparison, meat extract agar alone and in combination with dyes and indicators, and also the MacConkey medium were included in these tests. The results of three experiments, with three different, recently isolated typhoid and colon strains are summarized in Table 1. The experiments denoted by the same number were made at the same time in each case and were made in duplicate or triplicate.

Table 1 shows clearly that peptic digest mediums are slightly superior to veal infusion agar. These two again give constantly better growth of typhoid bacilli than the ordinary meat extract agar. The differences are not so marked for the colon bacillus.

All the selective mediums containing dyes or indicators inhibit the growth of typhoid bacilli to some degree. This inhibition is characteristic for some mediums like Drigalski-Conradi, Congo-red, and the MacConkey medium; it represents in the viable organisms a reduction of as high as 50%, and it is somewhat more marked on mediums made from veal infusion or on those prepared according to the original formula than on the peptic digest agar preparations. In the Drigalski-Conradi and Congo-red medium the inhibition of the colon bacilli is also striking.

Endo's medium prepared from veal infusion or peptic digests and particularly Kendall's modification show slight inhibition only. Among the brilliant green and malachite green mediums, the Teague medium is distinctly the best. It is of importance to emphasize in this connection that only a few preliminary trials were made to determine the

optimum concentration of the brilliant green in the various batches of agar used; therefore, it is not unlikely that results even better than those shown in Table 1 could have been obtained with Teague medium or with Krumwiede's formula. Considering the data recorded from this viewpoint, the superiority of Teague medium in comparison with the Robinson and Rettger's modification of Endo's medium, is even more striking.

TABLE 1

SHOWING THE AVERAGE NUMBER OF COLONIES WHICH GREW ON VARIOUS MEDIUMS IN EXPERIMENTS MADE WITH VARIOUS KNOWN DILUTIONS OF PURE CULTURES OF B. TYPHOSUS AND B. COLI, RESPECTIVELY

Medium and Indicator	Number of Colonies on Plates Inoculated with B. Typhosus 0.02 C C Experiments			Number of Colonies on Plates Inoculated with B. Coli 0.2 C C Experiments		
	1	2	3	1	2	3
1. Veal infusion, Witte's peptone agar	145	176	55	784	194	25
2. Veal infusion agar, Endo's medium, Robinson-Rettger's modification	112	140	46	680	181	24
3 Veal infusion agar, Congo-red indicator	94	124	48	685	196	20
4. Veal infusion agar, Drigalski-Conradi med.	53	88	—	280	122	—
5. Veal infusion agar, eosin-brilliant green medium of Teague	106	148	53	0	49	4
6. Veal infusion agar, malachite green-china blue medium of Bitter	85	144	—	47	79	—
1. Peptic digest agar	163	296	61	760	204	28
2. Peptic digest agar, Endo's medium, Robinson-Rettger's modification	154	178	47	660	201	25
3. Peptic digest agar, Kendall's modification	—	197	55	—	—	—
4. Peptic digest agar, Congo-red indicator	122	199	49	700	199	21
5. Peptic digest agar, Drigalski-Conradi med.	108	174	—	160	168	—
6. Peptic digest agar, eosin-brilliant green (Teague)	155	225	56	0	94	15
7. Peptic digest agar, malachite green-china blue (Bitter)	120	144	—	323	147	18
8. Peptic digest agar, brilliant green medium of Krumwiede	150	198	53	10	180	14
1. Meat extract (Liebig's) in Witte's peptone agar	105	107	30	725	184	19
2. Meat extract, Endo's medium, original formula	92	90	—	560	142	20
3. Meat extract, brilliant green, Andrade's indicator (Krumwiede)	67	87	24	6	56	4
1. MacConkey's medium	62	75	10	810	200	26

Series 2.—Dilutions of definite mixtures of pure broth cultures of B. typhosus and B. coli were spread on a similar set of mediums as reported in Series 1. The mixtures were used immediately to reduce as much as possible the known antagonistic effect of the colon bacillus on the typhoid bacillus.[20] The observations of Dreyer, Walker and Gibson have already indicated that the typhoid bacillus fails to grow on selective mediums when the proportion of typhoid to colon bacilli in the mixture was lower than 1 typhoid to 15 colon bacilli. We have, therefore, chosen mixtures which were within this range. Table 2 presents two of these tests.

[20] Nissle: Deutsch. med. Wchnschr., 1916, 42, p. 1181.

These experiments confirm the conclusions drawn from the first series and lend considerable support to the observations of Dreyer, Walker and Gibson. MacConkey's medium failed to indicate viable typhoid bacilli when the proportion in the mixtures was 1 typhoid to 15 colon bacilli; in higher proportions also a marked inhibition was noticeable (reduction to nearly 50 per cent.). Among the other mediums the Drigalski-Conradi medium (with crystal-violet) was slightly inhibitive in veal, more so in peptic digest agar. Congo-red, Teague's

TABLE 2

SHOWING THE NUMBER OF COLONIES OF B. TYPHOSUS AND B. COLI, RESPECTIVELY, WHICH GREW FROM MIXTURES MADE AT THE SAME TIME FROM THE SAME CULTURES SO AS TO CONTAIN 15 B. COLI TO EACH B. TYPHOSUS AND 5 B. COLI TO EACH B. TYPHOSUS

Medium and Indicator	Fifteen B. Coli Aerogenes to Each B. Typhosus in Each 0.02 C C		Five B. Coli (Stool) to Each B. Typhosus in Each 0.02 C C	
	Typhoid Colonies	Coli Colonies	Typhoid Colonies	Coli Colonies
1. Veal infusion agar	13	184	230	784
2. Veal infusion agar, eosin-brilliant green (Teague)	6	154	204	0
3. Veal infusion agar, Endo, Robinson and Rettger's modification	7	181	187	680
4. Veal infusion agar, Endo-Kendall's modification	11	126	200	690
5. Veal infusion agar and Congo-red indicator	12	180	180	675
6. Veal infusion agar, Drigalski-Conradi indicator	9	155	103	280
1. Peptic digest agar	17	204	226	760
2. Peptic digest agar and brilliant green-eosin (Teague)	13	200	216	0
3. Veal infusion agar, Endo, Robinson and Rettger's modification	9	224	173	660
4. Peptic digest agar, Endo-Kendall s modification	13	213	174	560
5. Peptic digest agar and Congo-red indicator	14	216	198	610
6. Peptic digest agar, Drigalski-Conradi indicator	4	219	120	160
MacConkey's medium	0	172	125	682

green, and the various modifications of Endo medium gave good results with slight inhibition. The slightly alkaline Endo medium—like Kendall's modification—showed a somewhat higher percentage of viable typhoid bacilli than the strongly alkaline (P_H 7.9-8.4) modification of Robinson and Rettger. Teague's medium proved its superiority only in the test plates which were heavily inoculated with the colon and typhoid bacilli mixture (5:1), probably on account of the ease with which the colonies could be identified and counted. On the other hand,

in the experiments with B. coli aerogenes-B. typhosus mixture, the advantages of the Teague indicator are apparent only in the peptic-digest agar.

Repeated experiments with different mixtures along identical lines, have proven (1) the value of peptic digest agar as a substitute for veal infusion agar, and (2) the excellent quality of the Teague's eosin-brilliant green medium.

Endo and Congo-red medium proved to be equally efficacious in some of these experiments, and as long as light seedings are chosen, they make reliable differential plates. The results with such mediums depend largely, however, on the tedious proper adjustment of the reaction and other factors, which are of minor importance in Teague's medium.

We omitted Krumwiede's brilliant green medium from these experiments because the quantitative results in Series I did not show any advantage over Teague's medium; for the purpose of cultural discrimination between B. coli and B. typhosus, Krumwiede's medium contains a less efficacious differential indicator in the decolorized acid fuchsin than the eosin in Teague's medium. Furthermore, the adjustment of the medium to the indicator is delicate, and slight differences are of greater consequence than in the eosin-brilliant green mixture.

Series 3.—To test our observations of Series 2 under the most natural conditions obtainable, we applied the modified Endo's and Teague's medium to our routine feces examination of experimental carriers and of a few human stools. These tests extended over two months and are particularly searching because (1) different lots of digest mediums and indicator solutions were used, and (2) the feces of the rabbit carriers contain (for numerous reasons, to be published shortly) typhoid bacilli, usually in small numbers only. The stool specimens (usually 10 feces balls) were emulsified in sterile ox-bile and incubated for 1 hour, as is customary in our laboratory. One plate of Endo's and one of Teague's medium was each charged with one 2 mg. loopful of the suspension and the drop evenly spread with a bent wire. The following results were obtained:

Total number of carrier stools examined on various days for typhoid bacilli, 472.

Endo medium, Robinson and Rettger's modification, 436 negative, 36, or 7.6% positive.

Brilliant green-eosin medium (Teague and Clurman), 419 negative, 53, or 11.2% positive.

Increased positive results, 3.6%.

Out of 53 typhoid stools only 67.9% were diagnosed by using Endo's medium (R. R. modification).

Proportion of Endo's medium to Teague's medium, 1: 1.47.

The foregoing tabulation of the stool examinations of carriers shows that it is possible to increase about 30% the positive isolations of the typhoid bacillus by means of the eosin-brilliant green medium. This percentage corresponds well with the one reported by Krumwiede, Pratt and MacWilliams with their brilliant green-acid fuchsin agar.

We fully agree with Teague and Clurman that, in the course of these comparative studies of the two mediums, we have repeatedly found typhoid bacilli on the green plates when the Endo plate gave only questionable colonies, or gave negative results. On the peptic digest-green agar, the typhoid colonies are very large, have a characteristic grayish-pink color and are easily distinguished from the other fecal bacteria which may develop. Aside from the usual roundish colonies, we noted repeatedly a characteristic "mutation type." The colony is large, irregularly shaped, not unlike a grape leaf and shows a network of ridges and furrows in the inside structure. Biochemically and serologically the organisms of these colonies are those of typhoid bacilli, which, as far as our observations have been completed, have not returned to the common type of colony formation inside of 24 generations.

To be sure, some experience is necessary to recognize the typhoid colonies, particularly when numerous B. coli aerogenes strains are present in the specimen of stool. But in our laboratory we have found that even beginners give preference to the Teague's medium for the purpose of differentiating B. typhosus from a variety of intestinal bacteria. Our experience with human stools on Teague's medium is as yet small. We examined daily the feces of six cases of typhoid convalescents by means of the eosin-brilliant green, Endo and malachite green-china blue medium. On several occasions both the Endo and malachite green mediums gave negative results, when the Teague medium revealed several typical colonies. In one series of stool examinations, the Endo medium failed repeatedly to show typhoid colonies even when the other mediums registered a relative abundance of viable B. typhosus. Experiments—to be reported in connection with other studies—demonstrated that the typhoid bacillus responsible for the infection from which the specimens of stool were collected, was only slightly alkali-resistant, in contrast with many other typhoid strains recently isolated. The optimum growth was found to be at an H-ion concentration of P_H 6.2 and multiplication was suppressed at P_H 7.8, which was the reaction of some of the Endo medium samples used for

the tests. This and other observations suggest the use of mediums with a neutral reaction, of which Teague's eosin-brilliant green medium is the one of choice.

Among the many advantages of peptic digests as substrata in Teague's medium, it is well to mention two: (1) the size of the typhoid colonies, and (2) the agglutinability of the typhoid bacillus isolated on such media.

TABLE 3

SHOWING THE NUMBER OF COLONIES WHICH GREW ON THE VARIOUS MEDIUMS IN TESTS MADE WITH VARIOUS KNOWN DILUTIONS OF PURE CULTURE OF B. PARADYSENTERIAE AND B. COLI, RESPECTIVELY

Medium and Indicator	Number of Colonies of B. Paradysenteriae to Each 0.02 C C Experiments		Number of Colonies of B. Coli to Each 0.02 C C Experiments	
	1	2	1	2
1. Veal infusion agar	85	72	509	115
2. Veal infusion agar, methylene blue-eosin, Holt-Harris, and Teague	87 small	68	497	77
3. Veal infusion agar, Endo's medium, Kendall's modification	86	60	501	93
4. Veal infusion agar, eosin-china blue (author's medium)	54	48	482	81
5. Veal infusion litmus lactose agar	58	52	488	82
6. Veal infusion Congo-red agar	68	48	471	72
7. Veal infusion agar, eosin-water blue (author's medium)	55	45	475	94
1. Peptic digest agar	82	98	518	119
2. Peptic digest agar, methylene blue-eosin	88	90	509	96
3. Peptic digest agar, eosin-china blue (author's medium)	67	72	499	88
4. Peptic digest agar, Endo's medium, Kendall's modification	77	58	520	83
5. Peptic digest litmus lactose agar	84	94	480	110
6. Peptic digest Congo-red agar	52	62	460	95
7. Peptic digest agar, eosin-water blue (author's medium)	68	44	478	63
MacConkey's medium	48	85	425	121
1. Meat extract agar	71	—	490	—
2. Endo's medium, modification of Kendall	52	—	468	—

Table 3 shows the average diameter of the various colonies grown on different mediums as determined by means of the microscope and a step micrometer. The figures noted in this table are self explanatory; one large colony supplies sufficient growth for several tentative agglutination tests and the inoculation of several carbohydrate mediums for further identification.

We have been impressed with the relatively small number of inagglutinable strains of B. typhosus which we encountered on Teague's medium, in contrast to the frequent finding of such strains on malachite green and some batches of Endo's medium. These conclusions are reached as the result of several thousands of tentative slides or com-

plete tube-macroscopic agglutination tests. In this connection we found that slightly inagglutinable strains can be made readily agglutinable by conducting the test at a temperature of 50-55 C., or by placing the plate, with the colony to be tested, at this temperature for from 3-4 hours. Using a highly potent goat serum in the dilutions of 1 : 1,000 and 1 : 5,000, together with a control dilution of normal goat serum of 1 : 20, we were able to give a tentative diagnosis in a very short time.

The results obtained by the slide agglutination tests were confirmed by fermentation tests in glucose and in mannite casein digest broth; and by the lead acetate test (P. P. Lévy and Pasteur v. Radot[21] and Kligler[22] in a 0.5% agar made with casein digest broth, by using small tubes, the expense of which is negligible. The additional labor of inoculating three tubes instead of one containing Russell's medium in its original form, or in Krumwiede's and Kohn's[23] modification, is well repaid. From our experience the results obtained on double or triple carbohydrate mediums for fermentation reactions are frequently misleading, because the colonies picked from green mediums are often impure.

FLUID ENRICHMENT MEDIUMS

It is not our purpose to discuss in detail the various fluid mediums which have been recommended for the enrichment of B. typhosus, and which one of us (J. E. Stickel) has investigated. Numerous tests were made with the main object of replacing the peptone solution, or the veal broth used in some of the fluid mediums, by our inexpensive peptic or tryptic digests. We have experimented with the brilliant green enhancement methods of Browning, Gilmour and Mackie,[24] of Robinson and Rettger,[3] of Krumwiede, Pratt and McWilliams,[7] of Tidy and Dunn,[2] and of Teague and Clurman.[4] For the present we feel justified in stating that for rabbit and monkey stools, at least, these mediums have in their original form or modified by the use of peptic or tryptic casein digests, proven unreliable and less efficacious than the direct plating methods on eosin-brilliant green agar. Even by following the advice of Dreyer, Walker and Gibson in using Endo plates, or by diluting the enriched stool culture and plating the dilutions on eosin-brilliant green agar as Teague has suggested, our results were not materially better when natural carrier stools of rabbits or monkeys

[21] Presse méd., Oct. 25, 1915, p. 420.
[22] Am. Jour. Pub. Health, 1917, 7, p. 805.
[23] Jour. Med. Research, 1917, 37, p. 225.
[24] Jour. Hyg., 1913, 13, p. 335.

were used. Artificial human stools frequently showed decided enrichment of the B. typhosus, and more especially when the dye dilutions were used in peptic digests. For rabbit stools we found sterile ox-bile to be an excellent enrichment fluid which, in some recent tests, increased considerably the percentage of positive typhoid findings in the stools. One of us will report on this phase of the problem soon.

The gelatin-Congo-red-brilliant green bromoform enhancement method of Teague and Clurman gave promising results with two human stools. Our experience being based largely on rabbit-carrier stools, is, however, limited and we shall reserve our final conclusions until we have had further opportunities to test with human material. The disadvantages of all the enrichment fluids is that a few more positive findings only are obtained at increased time, cost and labor. We are convinced that, in large comparative series, those apparently favorable results noted with fluid enrichment mediums are counterbalanced by the equally dependable and quicker results obtained by the use of peptic digest-eosin-brilliant green agar according to Teague and Clurman and modified by us.

The method of Morishima and Teague[25] suggested for the isolation of typhoid bacilli from urine has proven satisfactory in our study of experimental renal carriers. The nutrient broth recommended in the original formula can to advantage be replaced by peptic digests.

II. THE DETECTION AND IDENTIFICATION OF THE ORGANISMS OF THE DYSENTERY AND PARADYSENTERY GROUP BY MEANS OF SELECTIVE MEDIUMS

The cultural methods employed for discovering, isolating and identifying the B. dysenteriae present in dejecta are similar to those found useful in examining typhoid patients and for isolating B. typhosus from carriers. Three types of selective mediums have been used as plating substrata, Endo's medium in America, MacConkey's medium in England, and Drigalski-Conradi modified according to Lentz[26] in France and Germany, Denmark (Sonne) and India (Fraser). Recently, Congo-red-agar has been advocated by Lieberman and Acél[27] and Lomas[28] as a more selective medium than litmus lactose agar. As

[25] Jour. Infect. Dis., 1917, 21, p. 145.
[26] Handbuch. d. pathog. Mikroorgan., 1913, 3, p. 924.
[27] Deutsch. med. Wchnschr., 1914.
[28] Boht. Inst. noc. di Higiene de Alfonso XIII, 1915, 11, p. 193, abstracted in Bull. de l'Institut Pasteur, 1916, 14, p. 355.

a rule, primary isolation of the dysentery bacilli does not offer great difficulties because the organisms are usually exceedingly numerous in the mucous flakes of the characteristic dejecta of the acute disease, or during exacerbations. The conditions are entirely different, however, when samples of formed stool of suspected carriers, or stools of children suffering from summer diarrhea, are examined. A high percentage of negative findings is the rule, and only repeated, laborious plating of such specimens will give dependable results. The reports by TenBroeck[29] and others from the Boston Floating Hospital supply sufficient information on this point, so that no further comment is necessary.

One of us[30] (J. E. Stickel) examined, during 1915, fifty cases of infantile diarrhea, using the original Endo method for the isolation of the suspected bacteria with absolutely negative results. During 1916, a similar series of diarrheas was studied with the aid of litmus lactose agar plates, and nearly 80% of the intestinal infections could be diagnosed as "bacillary dysentery." These observations were responsible for the comparative tests which we present in the following paragraph.

The method of study and the character of the experiments were the same as already outlined in the paragraphs on "typhoid." Preliminary tests had shown that the B. paradysenteriae is sensitive to an alkaline reaction of the agar. Mediums were, therefore, chosen in which the indicator reacted best near the litmus neutral point; the optimum H-ion concentration varying between P_H 6.8 to 7.2. It was found that the original Endo formula, Russell's, and Robinson and Rettger's modification were unsuitable and had to be replaced by Kendall's modification. The latter medium does not contain as sensitive an indicator as the other preparations, but is useful in dysentery work.

Veal infusion-peptone and meat extract agar were again compared with peptic digest agar; the outcome of the tests decided in favor of the latter.

Methylene blue-eosin medium of Holt-Harris and Teague[31]—recently recommended for the isolation of typhoid bacilli—gave excellent results when the saccharose was omitted. For the purpose of more striking differentiation we recommend, instead of methylene blue, the use of water or china blue.[32] Only recently, isolated paradysenteriae strains not older than 2 or 3 weeks were used either singly or in mixtures for the study of the various mediums.

[29] Bost. Med. and Surg. Jour., 1915, 173, p. 280.

[30] Master Thesis, University of California, 1916.

[31] Jour. Infect. Dis., 1916, 18, p. 596.

[32] Preparation of Eosin-China Blue or Water Blue Agar: To 100 c.c. of melted peptic liver digest or tryptic beef heart digest agar with a reaction of P_H 6.8 to 7.0, neutral or very slightly alkaline to litmus, add 4 c c of a sterile 20% solution of milk sugar (Merck), heat for 15 minutes in the Arnold and then add 1-2 c c (somewhat less dye is required when American eosin instead of Grübler's or Merck's anilin red is used) of a 2% aqueous solution of yellowish water soluble eosin and 1 c c of a 5% aqueous solution of China blue (Grübler). Shake the mixture very carefully and pour plates. Instead of china blue use 1 c c of a 0.5% aqueous solution of water blue (Grübler) to 100 c c of agar containing lactose and eosin, as already stated above. The dye solutions, prepared with sterile distilled water, should be stored in the refrigerator.

EXPERIMENTAL DATA

Series 1.—In a series of experiments, veal agar as a substratum in methylene blue-eosin, eosin-china blue, eosin-water blue, Congo-red, Endo's medium (Kendall's modification), litmus lactose, and MacConkey's medium, was compared with peptic digest agar media of the same composition. The tests were made with various batches of mediums and were repeated in duplicates. We present in Table 4 the results of two experiments only.

Table 4 shows that peptic digests can advantageously be used for the isolation of dysentery bacilli; in some mixtures of these organisms the development of colonies was increased on the peptic digest mediums (Exper. 2). Furthermore, the size of the colonies is usually twice

TABLE 4

SHOWING THE NUMBER OF COLONIES OF VARIOUS B. PARADYSENTERIAE AND B. COLI WHICH GREW FROM MIXTURES MADE AT THE SAME TIME FROM THE SAME CULTURES

Medium and Indicator	Nine B. Coli to Each B. Paradysenteriae to Each 0.02 C C		Thirty B. Coli to Each B. Paradysenteriae to Each 0.02 C C	
	Dysentery Colonies	Coli Colonies	Dysentery Colonies	Coli Colonies
1. Veal infusion agar	10	55	8	229
2. Veal infusion agar, methylene blue-eosin, Holt-Harris, and Teague	8	44	8	210
3. Veal infusion agar, Endo's medium, Kendall's modification	9	61	4	233
4. Veal infusion agar, eosin-china blue (author's medium)	9	48	5	217
5. Veal infusion litmus lactose agar	5	35	6	210
6. Veal infusion Congo-red agar	12	65	4	224
1. Peptic digest agar	8	45	8	205
2. Peptic digest agar, methylene blue-eosin	8	44	7	222
3. Peptic digest agar, Endo's medium, Kendall's modification	12	42	5	233
4. Peptic digest agar, eosin-china blue	10	46	4	198
5. Peptic digest litmus lactose agar	7	33	5	221
6. Peptic digest Congo-red agar	8	66	4	236
MacConkey's medium	5	42	6	208

the customary one noted on veal agar plates. Table 3 gives detailed information relative to this point. Among the dye indicators the eosin-methylene blue medium showed less inhibition than any of the other preparations tested; the differences, however, are very slight, because most of the mediums chosen are apparently well suited for the isolation of dysentery bacilli. In this connection attention is called to the very good results obtained with MacConkey's medium. This observation is contrary to our previous findings with the typhoid bacillus; even though the colonies are small, differentiation is excellent, and practically all of the viable dysentery bacilli have grown into colonies.

Endo's medium, litmus lactose agar and Congo-red medium are only slightly inhibitive, and can be used advantageously for the differentiation of dysentery bacilli from fecal bacteria.

The addition of china blue or water blue to the eosin-agar of Holt-Harris and Teague produces as a rule a slightly more inhibitive medium than when methylene blue is used. The only advantage these two dyes possess over methylene blue is the power of striking differentiation of the nonlactose fermenting colonies from the lactose ferment ing fecal bacteria. Examination of such plates in reflected as well as in transparent light, permits the picking of colorless colonies more readily than on the methylene blue-eosin medium.

TABLE 5

Showing the Average Size of 20 Isolated Colonies of B. Typhosus, B. Paratyphosus A, B. Paradysenteriae and B. Coli on Various Mediums after 20-24 Hours' Incubation

Medium and Indicator	B. Paratyphosus	B. Typhosus	B. Paradysenteriae	B. Coli
	mm.	mm.	mm.	mm.
1. Veal agar, eosin-brilliant green	1.8-2.0	1.8-2.2	—	2.8-3.5
2. Veal agar, Endo, Robinson and Bettger's modification	1.6-2.4	1.0-1.5	—	2.2-3.0
3. Veal agar, Endo, Kendall's modification	1.3-1.8	1.3-1.8	1.1-1.4	2.5-2.9
4. Veal agar, Congo-red	1.4	1.3-1.5	1.6-1.8	2.4-3.1
5. Veal agar, Drigalski-Conradi	1.2	1.2	0.8-1.2	2.2-3.3 (No crystal violet)
1. Peptic digest agar, eosin-brilliant green	2.0-2.8	2.1-2.5	—	1.2-1.6 3.2-3.8 (B. coli aerogenes)
2. Peptic digest agar, Endo, Robinson and Rettger's modification	1.3-2.5	0.9-1.4	—	1.8-2.8
3. Peptic digest agar, Endo, Kendall's modification	1.6-2.0	1.6-2.0	2.2-2.8	2.5-3.9
4. Peptic digest Congo-red agar	1.1-1.2	1.3-1.8	2.3-3.8	2.8-4.0
5. Peptic digest agar, eosin-methylene blue	1.8-2.0	1.6-2.5	3.2-4.2	3.2-3.5
6. Peptic digest agar, Drigalski-Conradi	0.9	1.1-1.4	1.1-1.4	2.3-2.7
1. Meat extract brilliant-green agar, Krumwiede	0.4	0.6-1.2	—	1.4-2.0
2. Meat extract, Congo-red	0.8	0.4-1.0	0.8	1.6-2.2
3. Meat extract, Drigalski-Conradi	0.8	0.5-0.8	0.5	1.4
MacConkey's medum	—	0.5-1.0	0.8-1.3	0.8-1.5

Series 2.—A series of dilutions of definite mixtures of pure broth cultures of various strains of B. dysenteriae Shiga and B. paradysenteriae were spread on a set of agar mediums containing various indicators found to be reliable in Series 1. The proportion of the dysentery to the colon bacillus, in the mixtures used, varied between 1 dysentery to 50 colon bacilli. The dysentery bacillus was not found on any of the mediums employed, when present in the mixture, in a proportion less than 1 dysentery to 30 colon bacilli. Varying numbers of B. dysenteriae were recovered from mixtures of 1 dysentery to 5 or 9, 12 or 15, 25 or 30 colon bacilli. The data of two tests are presented in Table 5.

The results shown in Table 5 suggest the following conclusions:

Peptic digest agar with Congo-red indicators, methylene blue-eosin, china blue-eosin, Endo's medium (Kendall's modification), litmus lactose agar and MacConkey's medium, in the order mentioned, are suitable mediums for the isolation and selective differentiation of dysentery bacilli.

We tried to test these conclusions under the most natural conditions, by examining a number of stools of dysentery gall bladder carriers in rabbits. This method of study was unsatisfactory, however, because the infected animals eliminated the dysentery bacilli in large numbers and for short intervals only. All of the mediums compared gave equally good results.

TABLE 6

COMPARATIVE STOOL EXAMINATIONS SHOWING THAT PARADYSENTERY BACILLI INOCULATED FROM SUSPENSION OF FECES ARE INHIBITED ON ENDO'S MEDIUM (ORIGINAL FORMULA)

Patient, Character of Stool and Preparation of Material for Plating	Medium	Total Number of Colonies	Number of Paradysentery Colonies
A. H., liquid, considerable mucus	Peptic digest agar, sugar-free	148	12*
	Peptic digest agar, sugar-free, Endo's original formula	82	5
	Peptic digest agar, sugar-free, methylene blue-eosin (Holt-Harris, and Teague) without saccharose	72	20
	Peptic digest agar, sugar-free, Congo-red (Schmitz)	110	9
	Peptic digest agar, sugar-free, china blue eosin	60	16
	MacConkey's medium	91	14
B. B., watery, diarrheic stool strained through cotton	Peptic digest agar, sugar-free	1,262	98†
	Peptic digest agar, sugar-free, Endo's original formula	1,325	38
	Peptic digest agar, sugar-free, methylene blue-eosin	805	73
	Peptic digest agar, sugar-free, Congo-red	1,185	43
	Peptic digest agar, sugar-free, china blue-eosin	542	61
	MacConkey's medium	1,397	136
M. F., soft but formed stool, convalescent from mild dysentery, suspension shaken and strained through cotton	Peptic digest agar, sugar-free	123	37†
	Peptic digest agar, sugar-free, Endo's original formula	134	8
	Peptic digest agar, sugar-free, methylene blue-eosin	101	56
	Peptic digest agar, sugar-free, Congo-red	128	21
	Peptic digest agar, sugar-free, china blue-eosin	60	32

* Hiss-Y-Russell. † Flexner.

Thus far only three stool samples of paradysentery cases have been available for an actual test of the mediums with which we have experimented. The data collected are presented in Table 6.

The specimens examined came, unfortunately, from acute cases of paradysentery and contained the causative bacilli in abundance. The comparative examinations have therefore only a relative value, but they support our previous observations that the Endo medium (original formula) is inhibitive for the dysentery organisms. Furthermore, Congo-red mediums proved less reliable in these stool examinations than in the previously reported tests with mixtures of pure cultures. This difference is in part due to the heavy seeding of the plates and the diffusion of the acid Congo-red color into the surrounding of the lactose-fermenting colonies. Therefore, preference should be given to the mediums in which the dye is fixed to the colony or diffused only slightly and in which the type of colony can be recognized in reflected light. These requirements are fulfilled by the methylene blue- or china blue-eosin medium which, as these few practical tests indicate, are very slightly inhibitive, or not at all. The advantages of mediums with non-diffusible (methylene blue) or slightly diffusible (china blue) indicators over the other media thus far recommended, have also been noticed on scores of artificial dysentery stools which we have examined.

In addition to the advantages of the methylene blue-eosin agar over the Endo medium already enumerated by Holt-Harris and Teague, we feel justified in adding the following:

1. The expensive meat extract agar can be replaced by a cheap peptic liver digest agar. Sugar free or trypsinized beef heart digests give more striking differentiation of the dysentery colonies than those of the fecal bacteria. For routine work, however, the experienced can use satisfactorily the unfermented digests. On our modification of the methylene blue-eosin medium, the colonies are larger and are therefore more readily recognized.

2. The reaction of the agar does not require careful adjustment, because slight variations fail to affect the efficacy of the indicators. We found a neutral, or very slightly alkaline, reaction to litmus paper or on adjustment to an H-ion concentration of P_H 6.8 to 7.0 the optimum for our digest agar.

3. The plates remain unchanged when exposed to light, and in our experience they fail to deteriorate when kept at ice-chest temperature for over one week. Most of the other mediums, and particularly the Endo medium, are only dependable when fresh.

The use of methylene blue has, in our experience, only one disadvantage, namely, the inhibitive action of this dye on fecal cocci. As a

result of this inhibition, it frequently happens that the colony which is picked from the plate for further identification is not pure. On the other hand, china blue does not inhibit the cocci and the plates prepared with this dye permit of primary isolation of pure colonies.

For the final identification we use carbohydrate solution in sugar-free peptic digests and the agglutination test with polyvalent or monovalent dysentery sera. The detailed procedure will be found in our recent study on paradysentery in California.[33]

Experiments to selectively enrich the dysentery bacilli are still in progress. Unfortunately we have not been able to procure an additional supply of the dyes which have given thus far the most promising results.

CONCLUSIONS

Peptic liver digest agar is a better substratum for the isolation of organisms of the typhoid-dysentery group from stool and urine specimens than veal infusion or meat extract agar.

For the primary isolation of typhoid or paratyphoid organisms from stool and urine of patients or carriers, the eosin-brilliant green medium of Teague and Clurman can be highly recommended, when properly prepared with peptic digests. This solid medium permits the detection of a higher percentage of viable typhoid bacilli than any other one thus far introduced into bacteriologic technic.

Even though our evidence from actual cases is at present small, we consider the eosin-methylene blue medium of Holt-Harris and Teague, or our eosin-china blue medium prepared with peptic or tryptic digests, superior to Endo's medium, litmus lactose or Congo-red agar for the direct isolation and detection of dysentery bacilli from stool specimens.

[33] Meyer and Stickel, Calif. State Jour. Med., 1917, 15, p. 139.